

Award Number:
W81XWH-13-2-0016

TITLE:

Development of in Vivo Biomarkers for Progressive Tau Pathology after
Traumatic Brain Injury

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REPORT DATE:

February 2016

TYPE OF REPORT:

Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

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REPORT DOCUMENTATION PAGE				<i>Form Approved</i> OMB No. 0704-0188	
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1. REPORT DATE (DD-MM-YYYY) February 2016		2. REPORT TYPE Annual		3. DATES COVERED (From - To) 15 Jan 2015 - 14 Jan 2016	
4. TITLE AND SUBTITLE Development of in Vivo Biomarkers for Progressive Tau Pathology after Traumatic Brain Injury				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-13-2-0016	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) David L Brody Marc I Diamond				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Washington University 660 S Euclid Ave St Louis, MO 63110				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Athletes in contact sports who have sustained multiple concussive traumatic brain injuries are at high risk for delayed, progressive neurological and psychiatric deterioration ¹⁻⁹ . This syndrome is termed chronic traumatic encephalopathy (CTE) ^{1, 7, 10} , and is also known as dementia pugilistica ^{3, 11} or 'punch drunk' syndrome ^{9, 12} . US military personnel ^{13, 14} and others who have sustained multiple concussive traumatic brain injuries ¹⁵⁻¹⁷ may also be at risk for this condition. Currently, there are no methods to identify progressive tau pathology in living humans. Hypothesis: Aggregated forms of hyperphosphorylated tau protein formed acutely in the setting of traumatic brain injury can seed further aggregation of intracellular tau in nearby cells, leading to delayed propagation of tau pathology and neurodegeneration. Objective: To develop standardized, high-throughput blood and cerebrospinal fluid assays for aggregated forms of tau responsible for propagation of tau pathology after traumatic brain injury. Progress to date: To date, none of the attempts to model progressive tau pathology after repetitive concussive TBI in mice has been optimal. Ongoing efforts include development rotational acceleration concussive injury which can be repeated 20 times in the same mice, and subconcussive injuries. Continued progress towards increasing the sensitivity of cell-based assays for tau aggregation activity has been made, as well as antibody-based tau detection methods for blood samples.					
15. SUBJECT TERMS:					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER (include area code)

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1) INTRODUCTION: Athletes in contact sports who have sustained multiple concussive traumatic brain injuries are at high risk for delayed, progressive neurological and psychiatric deterioration ¹⁻⁹. This syndrome is termed chronic traumatic encephalopathy (CTE) ^{1, 7, 10}, and is also known as dementia pugilistica ^{3, 11} or ‘punch drunk’ syndrome ^{9, 12}. US military personnel ^{13, 14} and others who have sustained multiple concussive traumatic brain injuries ¹⁵⁻¹⁷ may also be at risk for this condition. Hyperphosphorylation and aggregation of tau protein are key pathological features of chronic traumatic encephalopathy, but at present they can only be observed post-mortem ^{1, 3, 6, 18-20}. Tau pathology has also been observed after single more severe traumatic brain injuries ²¹⁻²³. Currently, there are no methods to identify progressive tau pathology in living humans. The progressive aspect of chronic traumatic encephalopathy suggests that repetitive injuries may trigger an ongoing degenerative process similar to other diseases characterized by progressive tau pathology such as Alzheimer disease and frontotemporal dementia. A leading hypothesis regarding the progression of tau pathology in Alzheimer disease and frontotemporal dementia is that tau aggregates formed in one cell can propagate by exiting that cell and entering anatomically connected cells to induce tau aggregation in these cells ²⁴⁻³⁰. While the tau pathology in chronic traumatic encephalopathy is distinct from other diseases, the propagation model offers a new conceptual framework to test these ideas in chronic traumatic encephalopathy.

Hypothesis: Aggregated forms of hyperphosphorylated tau protein formed acutely in the setting of traumatic brain injury can seed further aggregation of intracellular tau in nearby cells, leading to delayed propagation of tau pathology and neurodegeneration.

Objective: To develop standardized, high-throughput blood and cerebrospinal fluid assays for aggregated forms of tau responsible for propagation of tau pathology after traumatic brain injury.

BODY

PROGRESS DURING THE REPORTING PERIOD:

TASK 1: *To assess extracts from the brains of tau transgenic mice subjected to experimental traumatic brain injury for tau aggregating activity using a cultured-cell based assay.*

Brody Laboratory

As noted in previous reports, the major challenge has been to reliably recapitulate progressive tau pathology triggered by repetitive concussive TBI in an animal model. During the reporting period, we took a novel approach to the problem:

It has been reported that heavy use of alcohol among male military personnel aged 18-25 is (32.2%), this is higher than civilian males of the same age (17.8) ³¹. Mild focal traumatic brain injury followed by acute alcohol exposure in rats has been reported to lead to an increase in local neuroinflammation ³². In transgenic mouse models of increased microgliosis tau pathology was exacerbated and spatial memory was impaired ³³. Because these studies were done separately no conclusions can be drawn about the correlation of TBI induced neuroinflammation and tau pathology. Therefore, we hypothesized that binge alcohol consumption after repetitive concussive traumatic brain injury would exacerbate the development of tau pathology. To test this hypothesis, we created an experimental paradigm of repetitive concussive brain injury and binge like alcohol consumption to explore the potential synergistic effects of both on the acceleration of the accumulation of pathological tau in tau transgenic mice. Specifically, we used an injury model developed previously ³⁴ that has been reported to exacerbate tau pathology ³⁵ combined with a well-established binge-like alcohol exposure paradigm called 'drinking in the dark' ^{36, 37}

Experimental design: 12 month old human tau transgenic mice were subjected to 5 concussive brain injuries or sham injuries over 9 days. Starting one week after the 5th injury, half of the mice were administered increasing grades of ethanol in water (5%, 10%, 15% 20%) over 6 months. Four days each week the ethanol solution was available to the mice for differing times each day, 2 hours the first day of the week, 2 hours the second day, 3 hours the third day, and four hours the fourth day. The volume consumed was measured

at the end of each drinking period. For assessment of tau pathology, mice were sacrificed after 6 months of chronic alcohol exposure, brains were collected and prepared for histology. Histological assessment of tau pathology was performed using stereology and software based analysis on brains labeled with the anti tau antibody AT8 (Figures 1-2), silver staining for neurodegeneration (Figure 3), and Iba1 labeling of activated microglia (Figure 4).

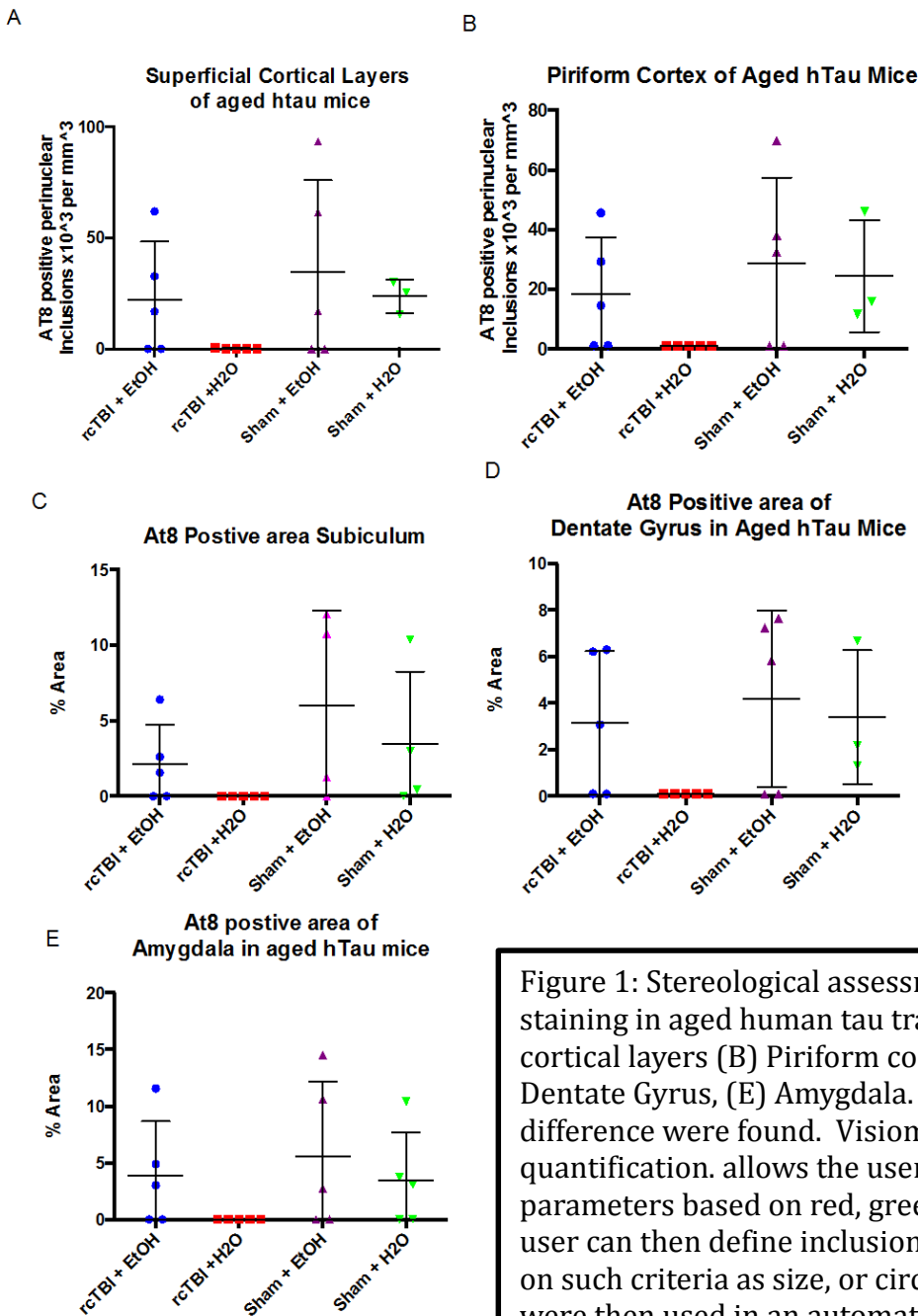


Figure 1: Stereological assessment of AT8 tau positive tau staining in aged human tau transgenic mice. (A) superficial cortical layers (B) Piriform cortex (C) subiculum, (D) Dentate Gyrus, (E) Amygdala. No statistically significant difference were found. Visiormorph Software was used for quantification. allows the user to define threshold parameters based on red, green, and blue color values, the user can then define inclusion and exclusion criteria based on such criteria as size, or circularity. These definitions were then used in an automated fashion. rcTBI: repetitive concussive traumatic brain injury. EtOH: binge alcohol exposure. No statistically significant differences between groups.

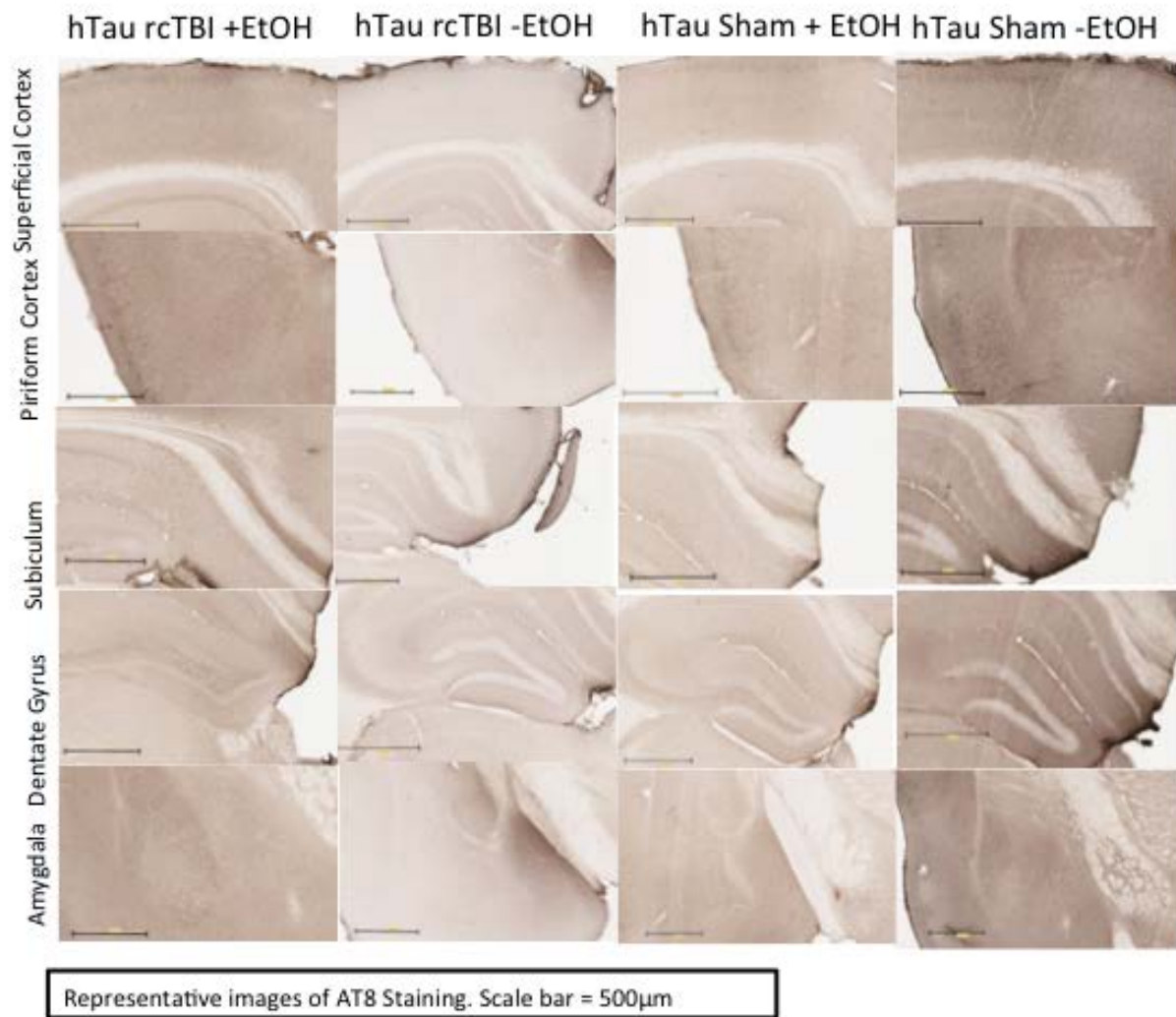


Figure 2:Representative tau immunohistochemistry

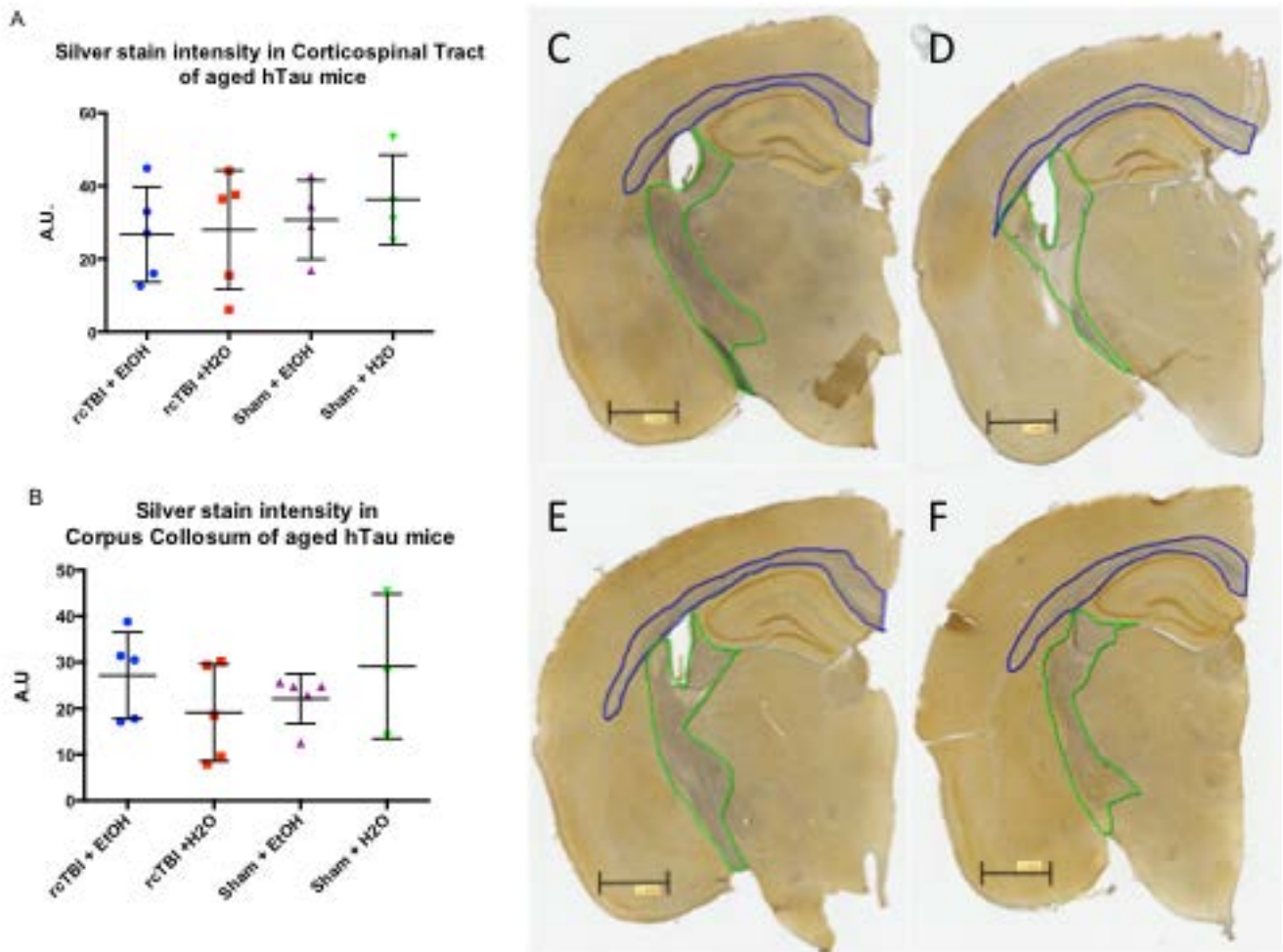


Figure 3: Silver stain analysis of white matter tracts in aged human transgenic mice. (A) Corticospinal tract. (B) Corpus Callosum. A.U. = Arbitrary Units. (C-F) Representative images of Silver Staining. (C) rcTBI + EtOH. (D) rcTBI + H2O. (E) Sham + EtOH. (F) Sham + H2O. Blue = Corpus Callosum. Green = Cortico Spinal Tract. Scale Bars = 1mm. No statistically significant differences between groups.

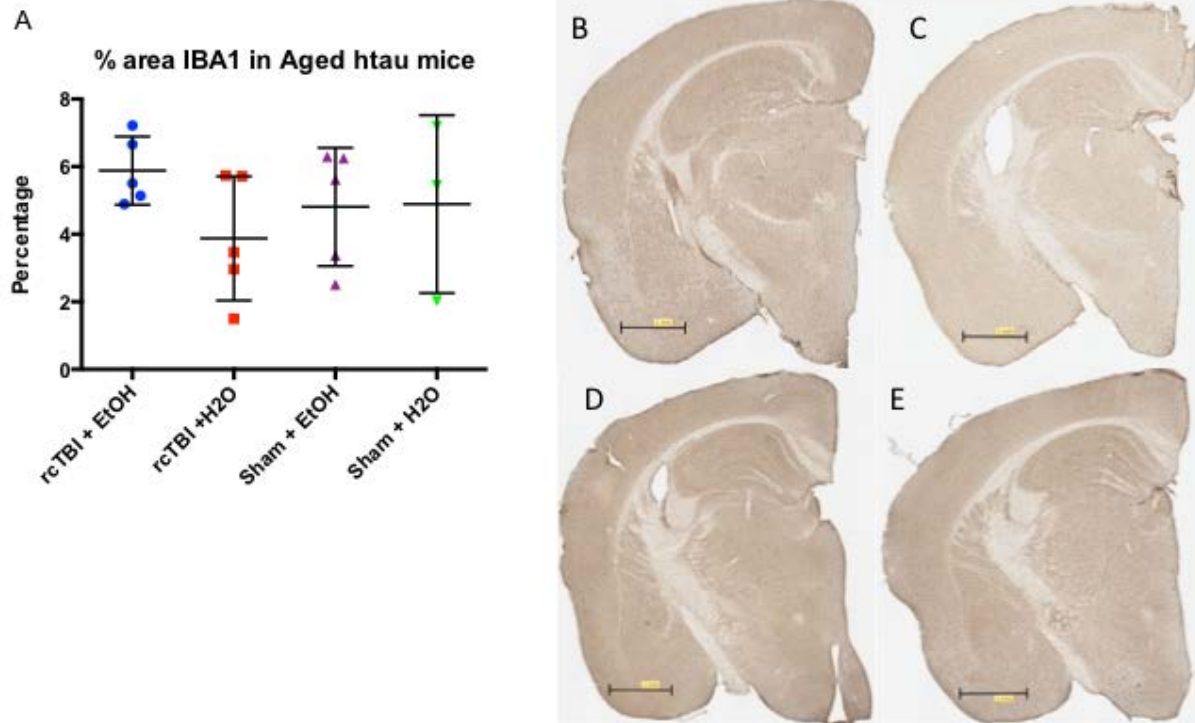


Figure 4: A. Microgliosis of aged human tau transgenic mice measured by percent of IBA-1 positive area, using visiomorph software. Entire brain slices were analyzed for global measurement. B-E. Exemplar images of IBA-1 staining. Scale bar = 1mm (B) rcTBI + EtOH. (C) rcTBI + H2O. (D) Sham + EtOH. (E) Sham + H2O Scale bar = 1mm. No statistically significant differences between groups.

Overall, the conclusion from these experiments was that ethanol exposure did not substantially affect the deposition of tau following repetitive concussive TBI. However, the experiments were not fully interpretable, because the concussive injuries did not cause the sort of substantial neurodegeneration (silver staining) nor microgliosis (Iba1 staining) as in previous experiments³⁸. It appears that the mice fully recovered from the repetitive concussive injuries over the 6 month period; notably, we did not observe the persistent neuroinflammation that has been reported by others, suggesting that differences between strains, ages, or genotypes of mice may play a role. Thus, additional experimental work on this topic will be required to assess the interaction between binge ethanol exposure and repetitive concussive TBI. Ultimately, more severe concussive injuries may be required to produce persistent pathological effects.

To date, it has been challenging to recapitulate progressive tau pathology following repetitive concussive traumatic brain injury in mice. Recently, a fundamentally new type of concussive TBI model for mice has been developed by our collaborators in the Wellington lab at the University of British Columbia called Closed Head Impact Model of Engineered Rotational Acceleration (CHIMERA).³⁹ We are now testing the hypothesis that rotational acceleration-induced TBI causes progressive tau pathology in 2 lines of transgenic mice. First, we commissioned construction, shipping and assembly of a CHIMERA device for our lab at Washington University.

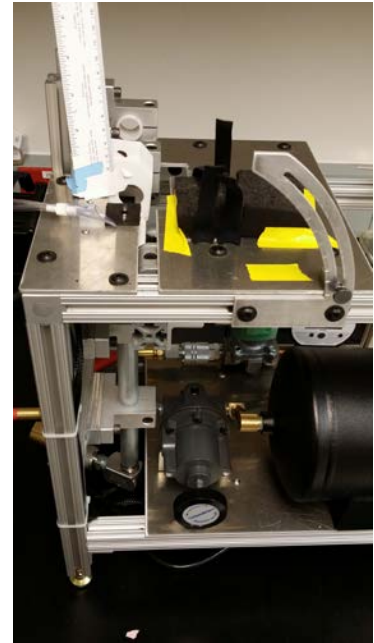
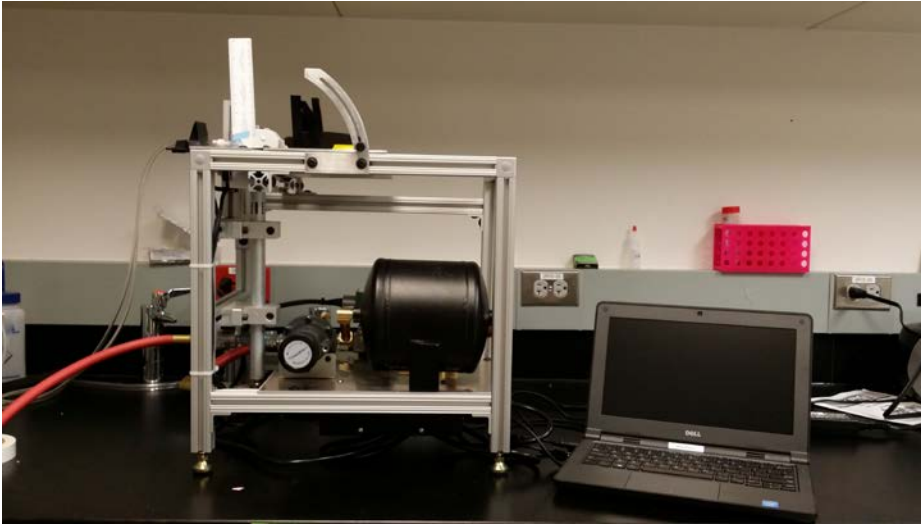


Figure 5: CHIMERA rotational acceleration injury device installed in the Brody lab.

After onsite training from University of British Columbia personnel, we have successfully implemented CHIMERA injuries in wild-type mice, recapitulating many of the basic findings in the original publication including delayed righting time and neuroscore deficits ⁴⁰.

Righting Time

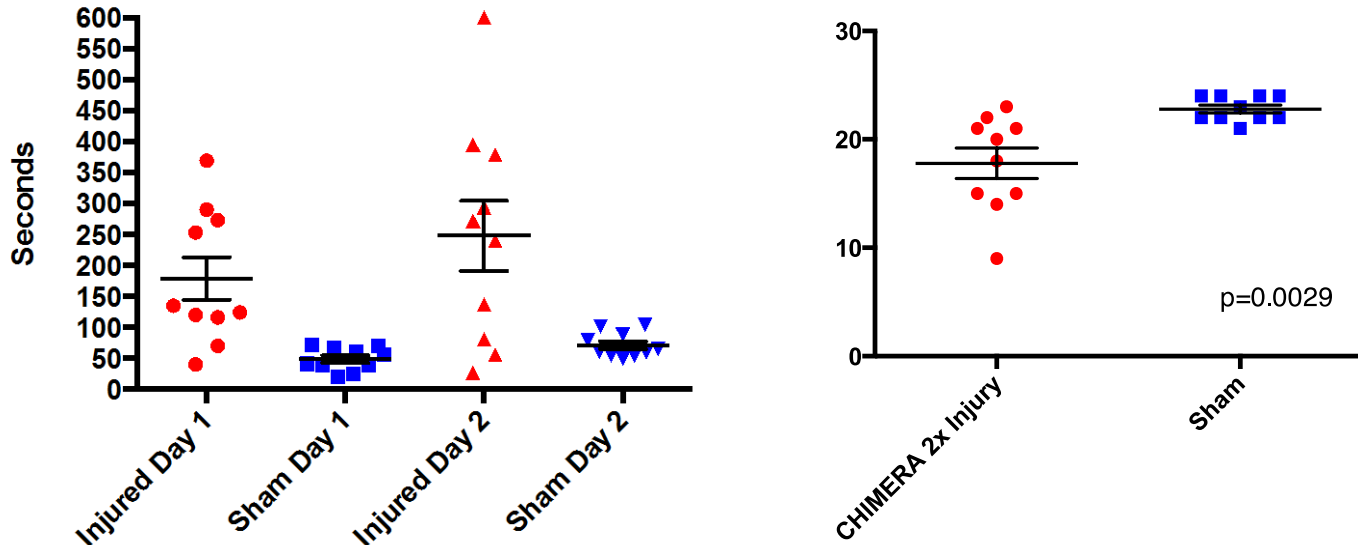


Figure 6: Our lab has reproduced two key behavioral aspects of the original publication, delayed righting time (left) and impaired neuroscore (right). Righting time is comparable in rodents to loss of consciousness in humans, and neuroscore comprises tests of motor function, exploratory behavior, startle reflex, and balance that are commonly impaired after concussion in humans.

Chimera Silver Stain Corpus Callosum

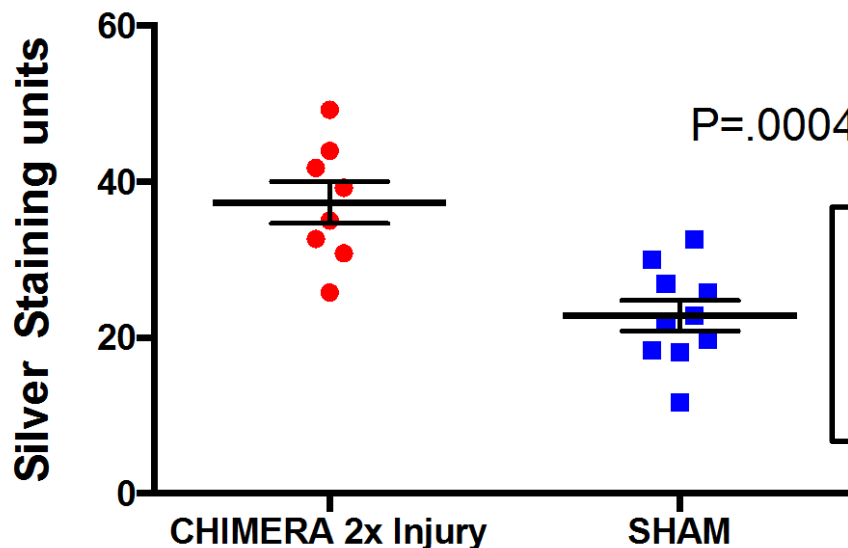


Figure 7: Silver stain analysis shows a statistically significant difference in silver stain impregnation, a marker of axonal degeneration between the CHIMERA 2x injury and sham groups. P value= 0.0004

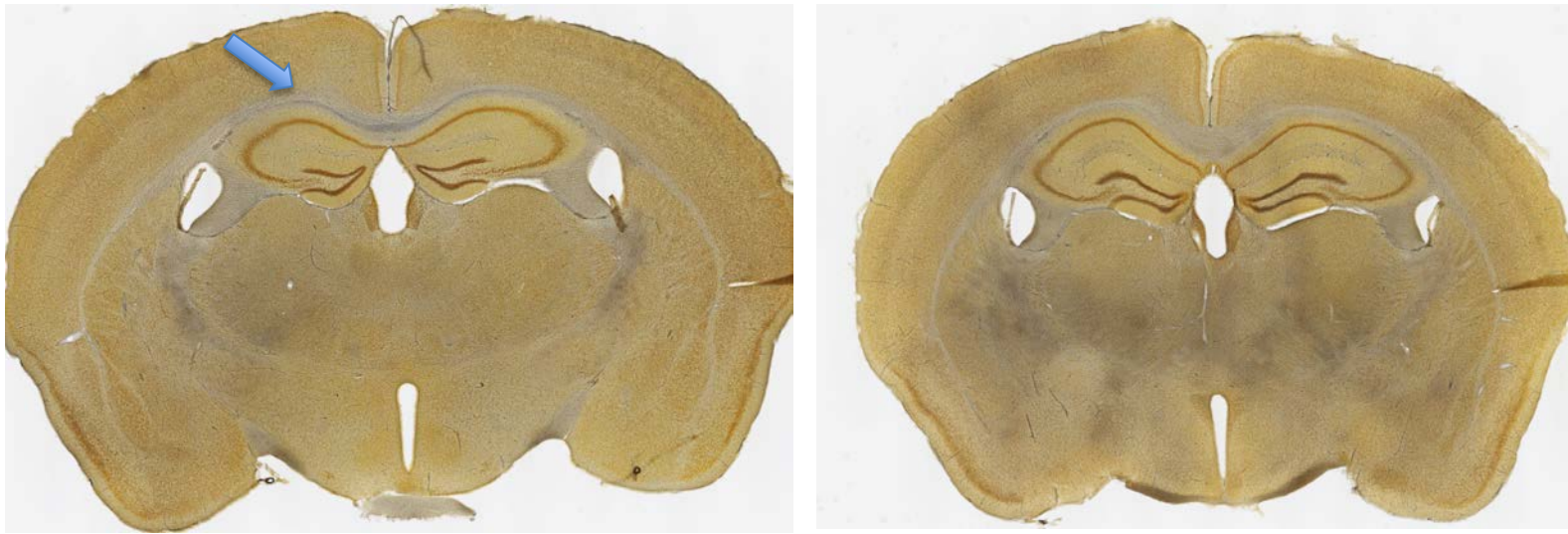


Figure 8: Representative silver stained images: Injured (Left) Sham (Right)

Furthermore, we established that a repeated injury paradigm with 2 injuries and 4 injuries 24 hours apart was logistically feasible and does not cause skull fractures or mortality. In our first experiment, we performed 1, 2 or 4 CHIMERA injury(s) or sham procedures spaced 24 hour apart in 16 week old P301S transgenic mice, and sacrificed them 24 hours after injury along with 4 sham (anesthesia only) treated littermate controls.

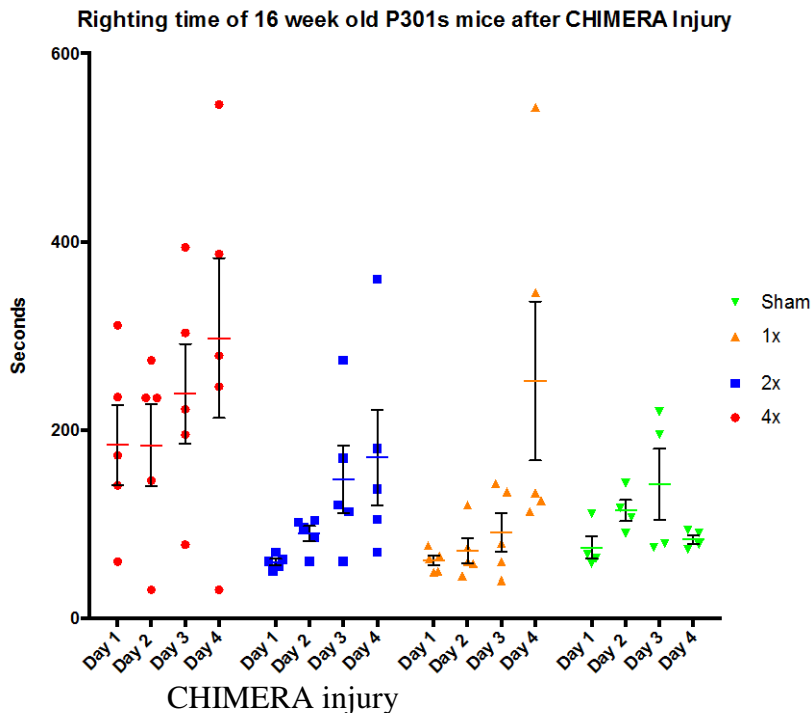
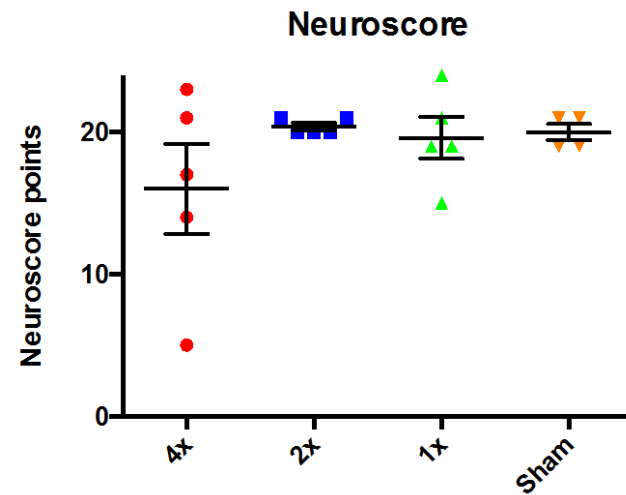


Figure 9:



Righting time and Neuroscore after

CHIMERA injury

We found that righting time continues to increase with each additional injury in the mice injured 2 and 4 times. The neuroscore used for wild-type mice may not be appropriate to detect deficits for this transgenic mouse line, as baseline impairment was observed compared to sham wild-type mice (see above). Instead, we chose to perform an elevated plus maze test, which highlights anxiety and risk taking behavior. Animals are placed on a plus shaped platform, two of the arms

are open, and two are closed, mice prefer to be in the closed arms, and any time spent in the open arms can be considered risk taking behavior, possibly associated with anxiety. In this particular injury paradigm with P301S mice, there was a stark difference in time spent in the open arm for the 2x, and 1x injury groups compared to sham, while the 4x group displayed minimal risk taking behavior. Taken with the behavior of the 2x and 1x injured mice, the 4x group may be displaying depressive like behavior rather than a lack of the risk taking behavior displayed by the other injured groups.

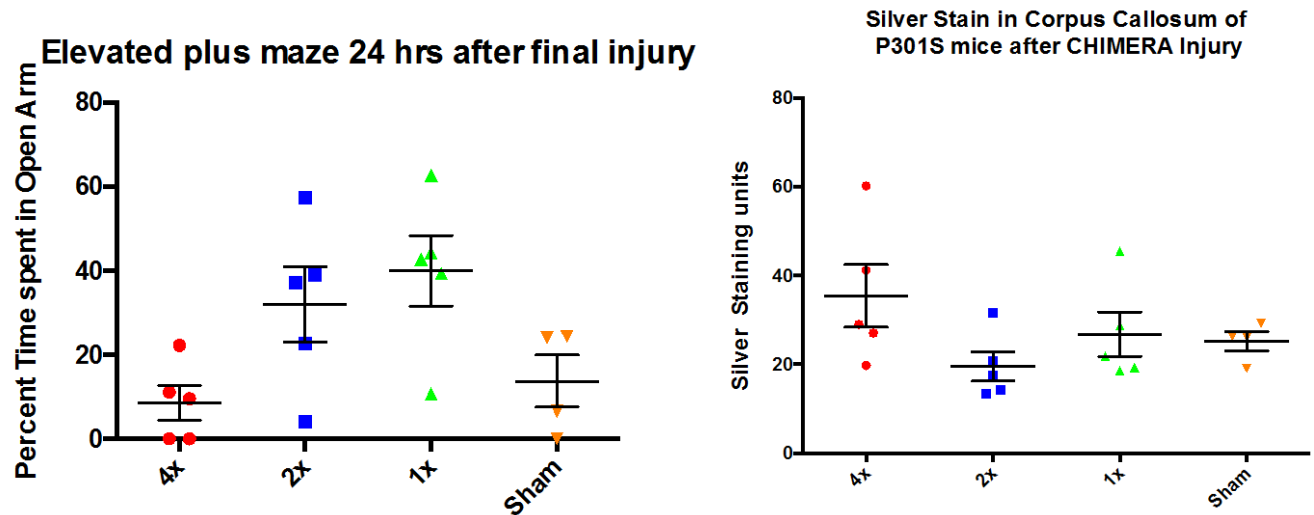


Figure 10: (Left) Elevated plus maze shows a contrast in risk taking behavior between groups. (Right) Silver Stain analysis of the corpus callosum.

Silver stain analysis of P301S mice after CHIMERA injury(s) was highly variable and did not show a statistically significant difference among groups. Previous studies in our lab have shown that in a different rcTBI model, silver stain intensity peaks at 7 days post injury³⁸, therefore, in future experiments we will assess silver staining at multiple time points after determine if this phenomenon holds true for the CHIMERA injury paradigm.

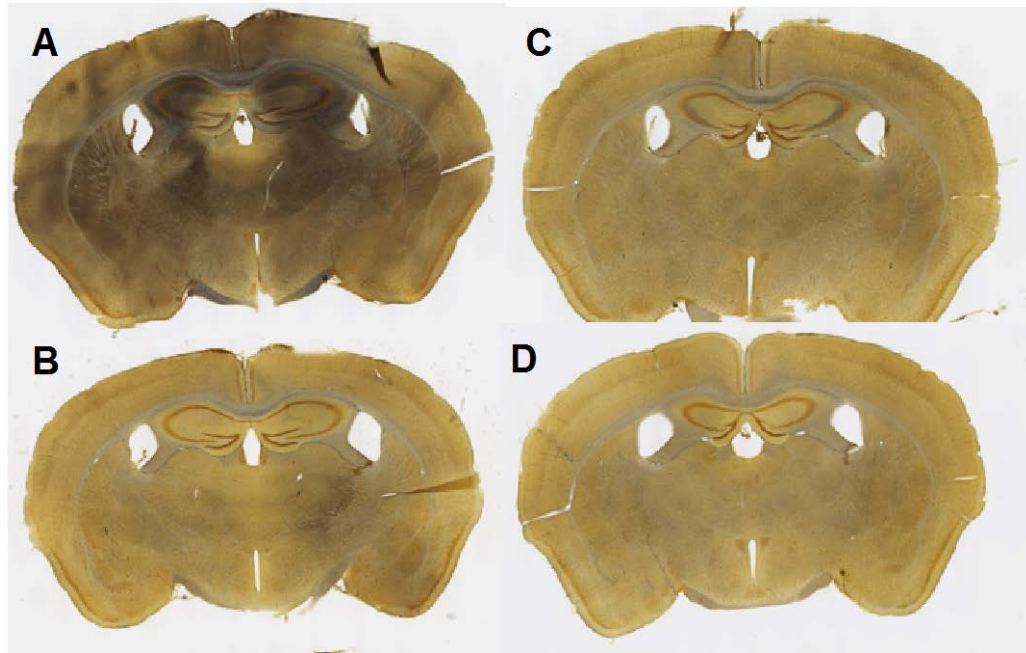


Figure 11: Exemplar images of Silver Staining in P301S mice after CHIMERA injuries. (A) 4 Injuries (B) 2 Injuries (C) 1 Injury (D) Sham injury

The main aim of the project is to recapitulate tau pathology following repetitive concussive TBI. Initial results with the anti-tau antibody AT8 have indicated some positive after 4 injuries but in an inconsistent fashion. We are currently quantifying the staining stereologically and performing additional antibody stains.

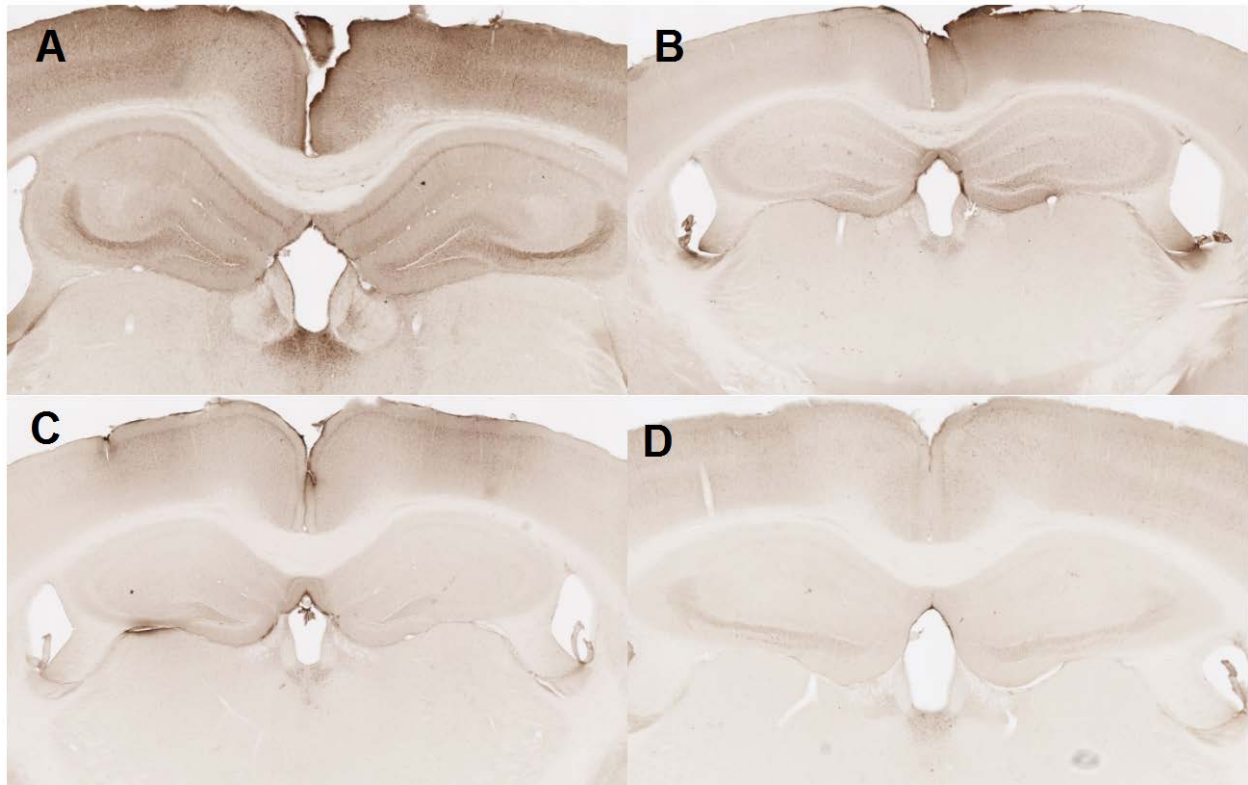


Figure 12: Representative images of AT8 staining in P301s mice after CHIMERA injuries. (A) 4 Injuries (B) 2 Injuries (C) 1 Injury (D) Sham Injury.

Diamond Laboratory

No progress to report, because of absence of funding for the past year.

TASK 2: *To determine whether mouse blood and cerebrospinal fluid tau aggregating activity quantitatively predict brain tau pathology and neurodegeneration in mice subjected to experimental traumatic brain injury*

Brody Laboratory

We have not performed any further tests of mouse blood or cerebrospinal fluid, since the optimal model for rcTBI-related tau pathology has not been determined.

Diamond Laboratory

No progress to report, because of absence of funding for the past year.

TASK 3: *To test whether antibodies that block tau aggregating activity in cultured cell-based assays also block tau pathology, neurodegeneration and behavioral deficits in mice subjected to experimental traumatic brain injury*

Brody and Diamond Laboratories

We have not made progress on this aim, because we are still working out the parameters of the experimental mouse models of TBI. Until we have a robust mouse model of TBI it will not be feasible to test antibody therapies.

TASK 4: *To develop an antibody-based assay for tau aggregating activity*

Brody Laboratory

We have not performed any experiments related to antibody-based assays during this award period.

Diamond Laboratory

No progress to report, because of absence of funding for the past year.

4) **PROBLEM AREAS:** We continue to work to develop mouse models of TBI that recapitulate individual aspects of human TBI-related neurodegeneration, including progressive tau pathology, atrophy, and tau seeding activity.

5) **WORK TO BE PERFORMED IN THE NEXT PERIOD:** During the next 12 months we expect to perform the following:

Brody Laboratory

- 1) Extensive testing of CHIMERA concussive injuries in hTau mice. Specifically, we will test single concussive injuries and up to 20 daily concussive injuries. We will determine the rotational acceleration threshold for concussive injury defined as prolonged righting time more than 2 standard deviations above the mean for sham injured mice recovering from anesthesia. We will then also test up to 20 sub-concussive injuries. hTau mice will be randomized to sham, subconcussive, or concussive injuries. Behavioral testing, histological analysis, and blood/CSF tau seeding activity will be assessed in a blinded fashion at multiple time points up to 12 months after injury.
- 2) Comparison of advanced MRI scans in mice to scans in human CTE tissue obtained as part of a separately funded NIH U01 grant. If promising, we will consider modifying the aims of the current grant to include imaging-based biomarkers as well as blood and CSF-based biomarkers. Specifically, locally disrupted axonal integrity and gliosis may be important signatures of CTE even in the absence of frank tau pathology.

Diamond Laboratory

If funding can be restored, based on transfer of grants from Washington University in St. Louis to UT Southwestern, we will continue to progress development of diagnostic tests to detect and characterize small amounts of tau aggregates. We will also apply the biosensor system to analyze tau aggregates within brain of Tg4510 tauopathy mice, and attempt to determine the levels of seeding activity in the periphery in these animals.

ADMINISTRATIVE COMMENTS:

It is likely that the Brody lab will require a no-cost extension for this grant.

The Diamond lab has not received funds for this grant since transferring to UT Southwestern. This has precluded further work on the project. Work will resume when funds are restored.

KEY RESEARCH ACCOMPLISHMENTS

- 1) Determination that initially proposed approaches to modeling repetitive concussive traumatic brain injury-induced tau pathology are not likely to yield a useful animal model for the development and validation of fluid biomarkers.
- 2) Determination that combined injury + binge ethanol in mouse models does not cause tau pathology.
- 3) Initial characterization of a more biophysically realistic rotational acceleration concussive TBI model for mice, with potential to perform up to 20 daily concussive or subconcussive injuries.

- 4) Development of improved cellular seeding assays for tau aggregation activity in fixed tissue
- 5) Development of a microfluidic approach to detect tau aggregates using an antibody-based detection system.

REPORTABLE OUTCOMES

None

CONCLUSION

While the initial investigations performed have not turned out as expected, we are making substantial progress and have several innovative new directions planned for the next year. The problem of diagnosis and serial assessment for tau-related pathology after repetitive concussive TBI is a critical one, and continued intensive study of the topic is warranted.

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